

Antigenic Variation of Avian Influenza A(H5N6) Viruses, Guangdong Province, China, 2014–2018

Appendix 1

Appendix 1 Table 1. Epidemiologic surveillance of H5, H7, and avian influenza A viruses, Guangdong province, China, January 2013–October 2018*

| Time | No. tested samples | FluA+, no. (%) | H5/FluA+, no. (%) | H7/FluA+, no. (%) |
|-----------|--------------------|----------------|-------------------|-------------------|
| Jan 2013 | 108 | 51 (47.22) | 6 (11.76) | 0 (0) |
| Feb 2013 | 81 | 23 (28.4) | 1 (4.35) | 0 (0) |
| Mar 2013 | 126 | 38 (30.16) | 4 (10.53) | 0 (0) |
| Apr 2013 | 230 | 74 (32.17) | 23 (31.08) | 0 (0) |
| May 2013 | 339 | 72 (21.24) | 1 (1.39) | 0 (0) |
| June 2013 | 182 | 15 (8.24) | 1 (6.67) | 0 (0) |
| July 2013 | 184 | 41 (22.28) | 13 (31.71) | 0 (0) |
| Aug 2013 | 256 | 66 (25.78) | 1 (1.52) | 0 (0) |
| Sept 2013 | 194 | 47 (24.23) | 7 (14.89) | 0 (0) |
| Oct 2013 | 196 | 51 (26.02) | 21 (41.18) | 1 (1.96) |
| Nov 2013 | 261 | 75 (28.74) | 15 (20) | 0 (0) |
| Dec 2013 | 656 | 242 (36.89) | 61 (25.21) | 58 (23.97) |
| Jan 2014 | 1,978 | 457 (23.1) | 56 (12.25) | 136 (29.76) |
| Feb 2014 | 1,800 | 464 (25.78) | 44 (9.48) | 160 (34.48) |
| Mar 2014 | 1,499 | 437 (29.15) | 73 (16.7) | 169 (38.67) |
| Apr 2014 | 1,436 | 328 (22.84) | 45 (13.72) | 94 (28.66) |
| May 2014 | 852 | 172 (20.19) | 27 (15.7) | 37 (21.51) |
| June 2014 | 489 | 147 (30.06) | 20 (13.61) | 31 (21.09) |
| July 2014 | 403 | 109 (27.05) | 32 (29.36) | 21 (19.27) |
| Aug 2014 | 403 | 126 (31.27) | 15 (11.9) | 7 (5.56) |
| Sept 2014 | 386 | 155 (40.16) | 6 (3.87) | 0 (0) |
| Oct 2014 | 401 | 151 (37.66) | 29 (19.21) | 3 (1.99) |
| Nov 2014 | 953 | 336 (35.26) | 70 (20.83) | 39 (11.61) |
| Dec 2014 | 1,377 | 548 (39.8) | 91 (16.61) | 59 (10.77) |
| Jan 2015 | 3,776 | 987 (26.14) | 140 (14.18) | 283 (28.67) |
| Feb 2015 | 3,279 | 655 (19.98) | 63 (9.62) | 235 (35.88) |
| Mar 2015 | 3,206 | 464 (14.47) | 47 (10.13) | 88 (18.97) |
| Apr 2015 | 2,689 | 526 (19.56) | 76 (14.45) | 27 (5.13) |
| May 2015 | 2,236 | 365 (16.32) | 37 (10.14) | 23 (6.3) |
| June 2015 | 934 | 139 (14.88) | 22 (15.83) | 6 (4.32) |
| July 2015 | 623 | 117 (18.78) | 18 (15.38) | 0 (0) |
| Aug 2015 | 758 | 119 (15.7) | 17 (14.29) | 2 (1.68) |
| Sept 2015 | 617 | 105 (17.02) | 13 (12.38) | 2 (1.9) |
| Oct 2015 | 636 | 121 (19.03) | 26 (21.49) | 0 (0) |
| Nov 2015 | 1,407 | 361 (25.66) | 62 (17.17) | 2 (0.55) |
| Dec 2015 | 2,080 | 644 (30.96) | 122 (18.94) | 9 (1.4) |
| Jan 2016 | 2,523 | 743 (29.45) | 161 (21.67) | 50 (6.73) |
| Feb 2016 | 2,186 | 537 (24.57) | 81 (15.08) | 52 (9.68) |
| Mar 2016 | 2,203 | 611 (27.73) | 109 (17.84) | 84 (13.75) |
| Apr 2016 | 1,755 | 342 (19.49) | 55 (16.08) | 12 (3.51) |
| May 2016 | 1,867 | 430 (23.03) | 54 (12.56) | 17 (3.95) |
| June 2016 | 625 | 85 (13.6) | 11 (12.94) | 3 (3.53) |
| July 2016 | 596 | 105 (17.62) | 7 (6.67) | 2 (1.9) |
| Aug 2016 | 750 | 132 (17.6) | 15 (11.36) | 0 (0) |
| Sept 2016 | 572 | 97 (16.96) | 19 (19.59) | 1 (1.03) |
| Oct 2016 | 712 | 99 (13.9) | 4 (4.04) | 4 (4.04) |
| Nov 2016 | 1,852 | 421 (22.73) | 52 (12.35) | 27 (6.41) |
| Dec 2016 | 2,590 | 686 (26.49) | 81 (11.81) | 206 (30.03) |
| Jan 2017 | 3,790 | 708 (18.68) | 58 (8.19) | 260 (36.72) |
| Feb 2017 | 3,341 | 672 (20.11) | 31 (4.61) | 247 (36.76) |

| Time | No. tested samples | FluA+, no. (%) | H5/FluA+, no. (%) | H7/FluA+, no. (%) |
|-----------|--------------------|----------------|-------------------|-------------------|
| Mar 2017 | 3,062 | 507 (16.56) | 33 (6.51) | 131 (25.84) |
| Apr 2017 | 1,910 | 355 (18.59) | 59 (16.62) | 101 (28.45) |
| May 2017 | 1,960 | 286 (14.59) | 44 (15.38) | 38 (13.29) |
| June 2017 | 599 | 55 (9.18) | 6 (10.91) | 12 (21.82) |
| July 2017 | 632 | 47 (7.44) | 17 (36.17) | 6 (12.77) |
| Aug 2017 | 581 | 90 (15.49) | 10 (11.11) | 0 (0) |
| Sept 2017 | 631 | 94 (14.9) | 13 (13.83) | 2 (2.13) |
| Oct 2017 | 809 | 168 (20.77) | 37 (22.02) | 0 (0) |
| Nov 2017 | 1,831 | 501 (27.36) | 101 (20.16) | 1 (0.2) |
| Dec 2017 | 1,758 | 412 (23.44) | 100 (24.27) | 11 (2.67) |
| Jan 2018 | 2,164 | 561 (25.92) | 134 (23.89) | 22 (3.92) |
| Feb 2018 | 1,892 | 324 (17.12) | 62 (19.14) | 8 (2.47) |
| Mar 2018 | 1,751 | 239 (13.65) | 32 (13.39) | 5 (2.09) |
| Apr 2018 | 1,776 | 262 (14.75) | 35 (13.36) | 1 (0.38) |
| May 2018 | 1,929 | 283 (14.67) | 27 (9.54) | 0 (0) |
| June 2018 | 655 | 69 (10.53) | 11 (15.94) | 0 (0) |
| July 2018 | 705 | 111 (15.74) | 28 (25.23) | 0 (0) |
| Aug 2018 | 656 | 141 (21.49) | 31 (21.99) | 0 (0) |
| Sept 2018 | 791 | 237 (29.96) | 58 (24.47) | 0 (0) |
| Oct 2018 | 933 | 160 (17.15) | 51 (31.88) | 0 (0) |
| Total | 8,9418 | 19,398 (15.73) | 2,932 (11.08) | 2,795 (6.28) |

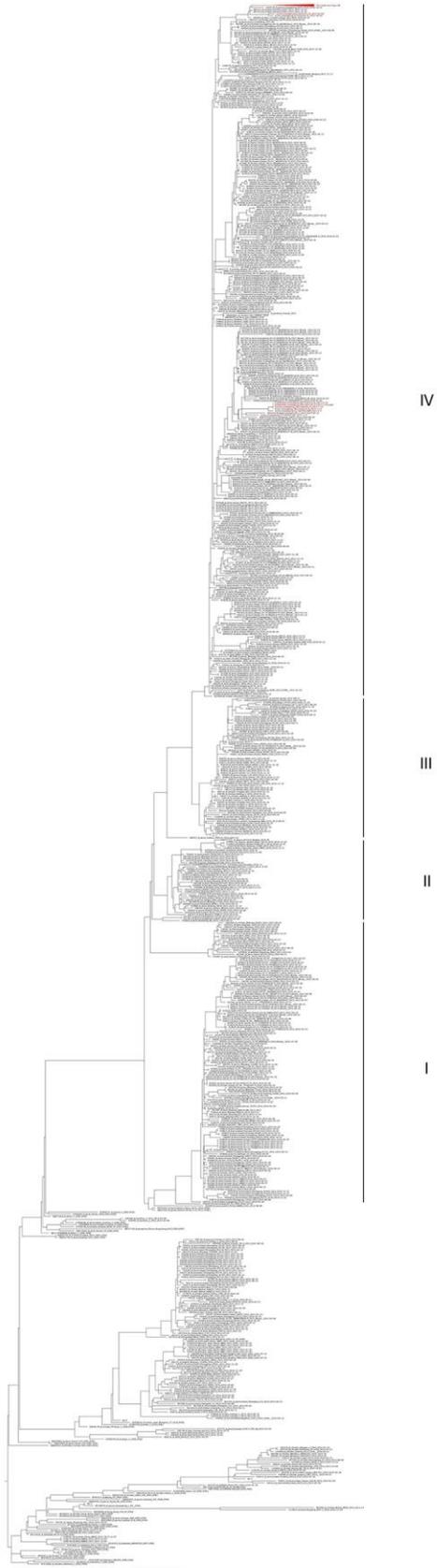
*Flu A+, type A avian influenza viruses; H5, H5 subtype avian influenza virus; H7, H7 subtype avian influenza virus.

Appendix 1 Table 2. Amino acid substitution of the hemagglutinin protein of influenza A(5N6) viruses from human and environment compared with vaccine strain A/Chicken/Guizhou/4/2013 (Re-8) (H3 numbering)

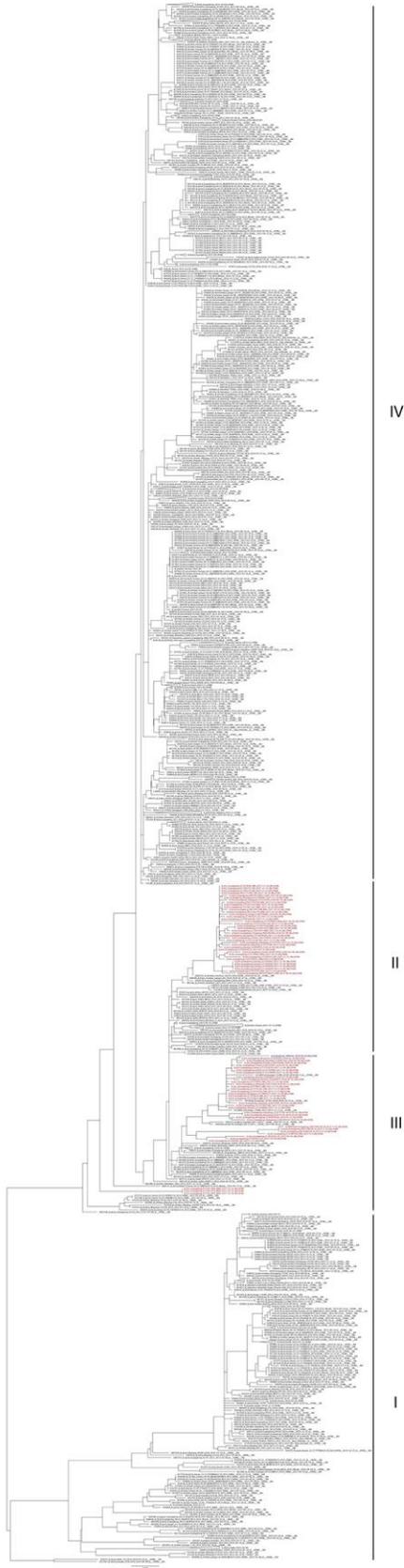
| Molecular feature or amino acid substitution | Amino acids of viruses (no.) | | |
|--|------------------------------|-----------|------------------------------------|
| | Re-8 (1) | Human (1) | Environment (72) |
| Multibasic cleavage site | | | |
| PLRERRRKR↓G | | | |
| Amino acid substitution | | | |
| R50K | R | K | K (67), R(5) |
| D63N | D | N | D (47), N (25) |
| R81S | R | S | R (26), S (46) |
| S94A/T | S | A | A (68), T (4) |
| L122Q | L | Q | L (7), Q (65) |
| S125R/K | S | R | R (57), K (2), S (13) |
| P128S | P | S | S (59), P (13) |
| D129N/S | D | N | N (69), S (3) |
| D130bdeletion/E/T | D | - | 65 (-), E (5), T (2) |
| T131S | T | T | T (70), S (2) |
| L133 deletion/S | L | S | 7 (-), S (65) |
| A137T | A | A | T (1), A (71) |
| A138S | A | A | S (1), A (71) |
| Q142K | Q | Q | Q (67), K (5) |
| M144V | M | V | V (70), M (2) |
| P145A | P | A | A (59), P (13) |
| I155T | I | T | T (72) |
| N158S | N | N | S (1), N (71) |
| T160A | T | A | A (72) |
| R173G/K | R | K | G (5), K (56), |
| S185P | S | S | P (3), S (69) |
| N187S | N | S | S (60), N (12) |
| A188V | A | A | V (1), A (71) |
| A189E | A | E | E (71), A (1) |
| T192A | T | T | A (8), T (64) |
| N193D/K/T/N | N | D | D (26), K (10), T (1), N (35) |
| T199A/I | T | T | T (70), I (1), A (1) |
| R227S/C/Q/G | R | R | S (4), C (2), Q (5), R (58), G (3) |
| K238R | K | R | R (62), K (10) |
| V260I | V | I | I (67), V (5) |
| K262T | K | T | T (67), K (5) |
| M272I | M | I | I (63), M (9) |
| H276K/N/Q/S | H | N | K (2), N (60), Q (1), S (5), H (4) |
| N278S | N | N | N (71), S (1) |
| N323S | N | S | S (72) |

*-, deletion.

A



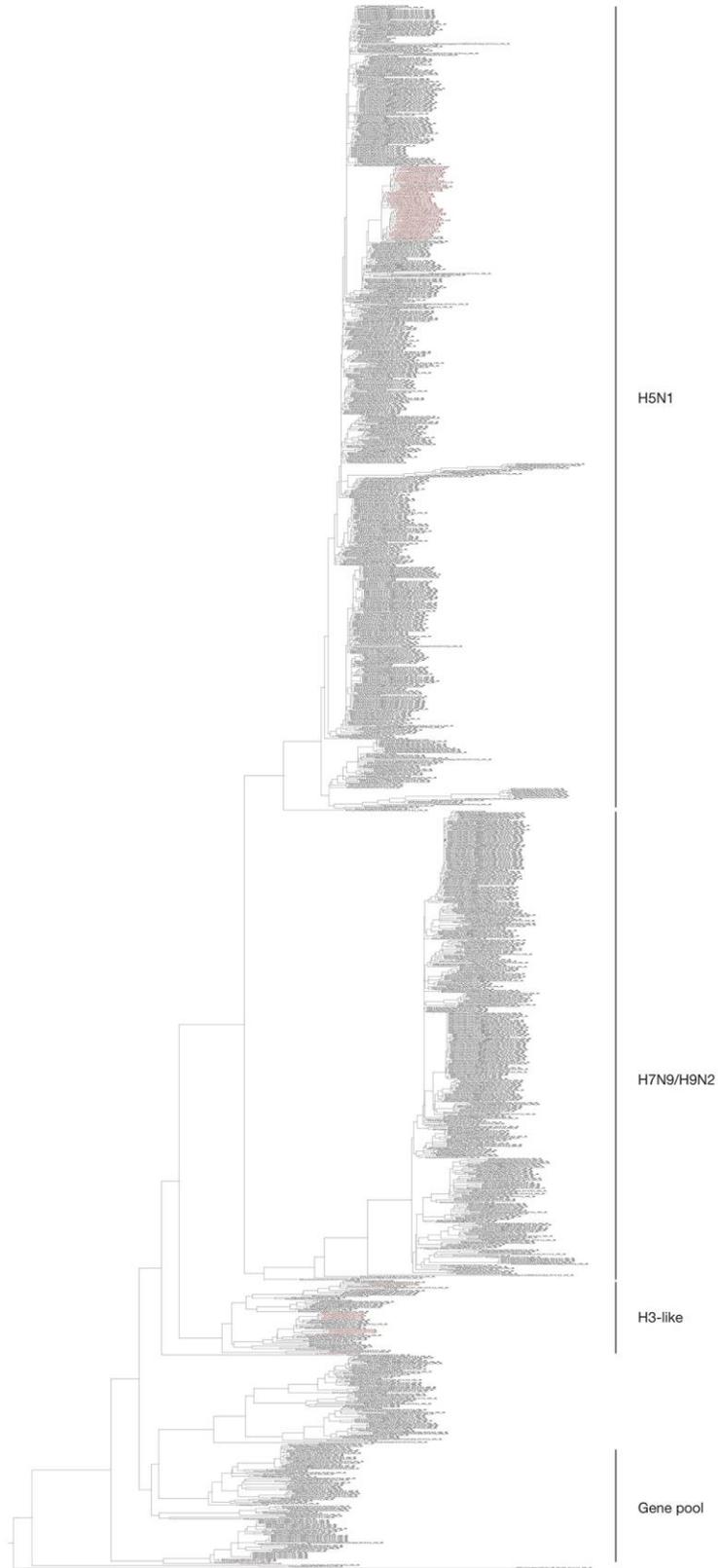
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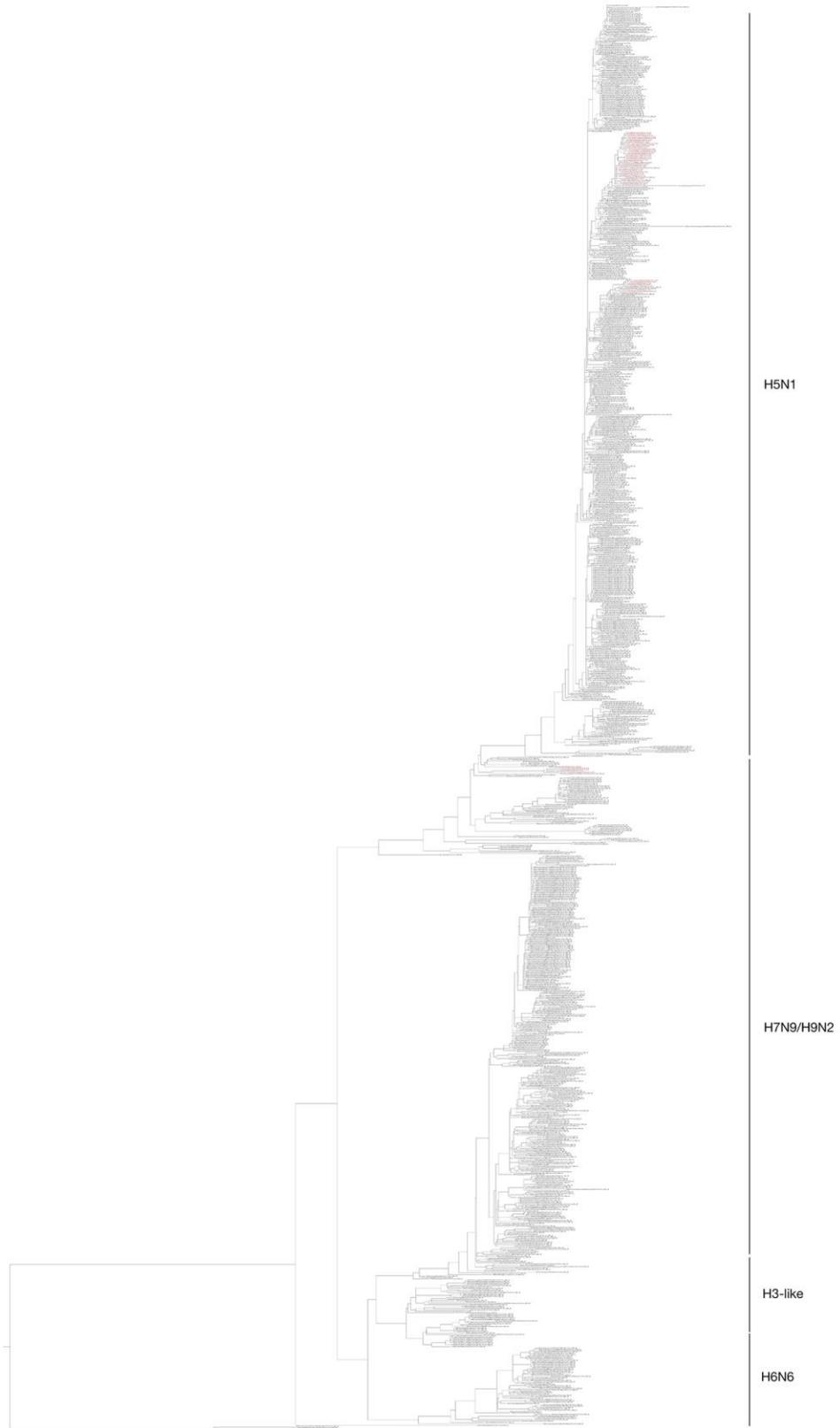
C



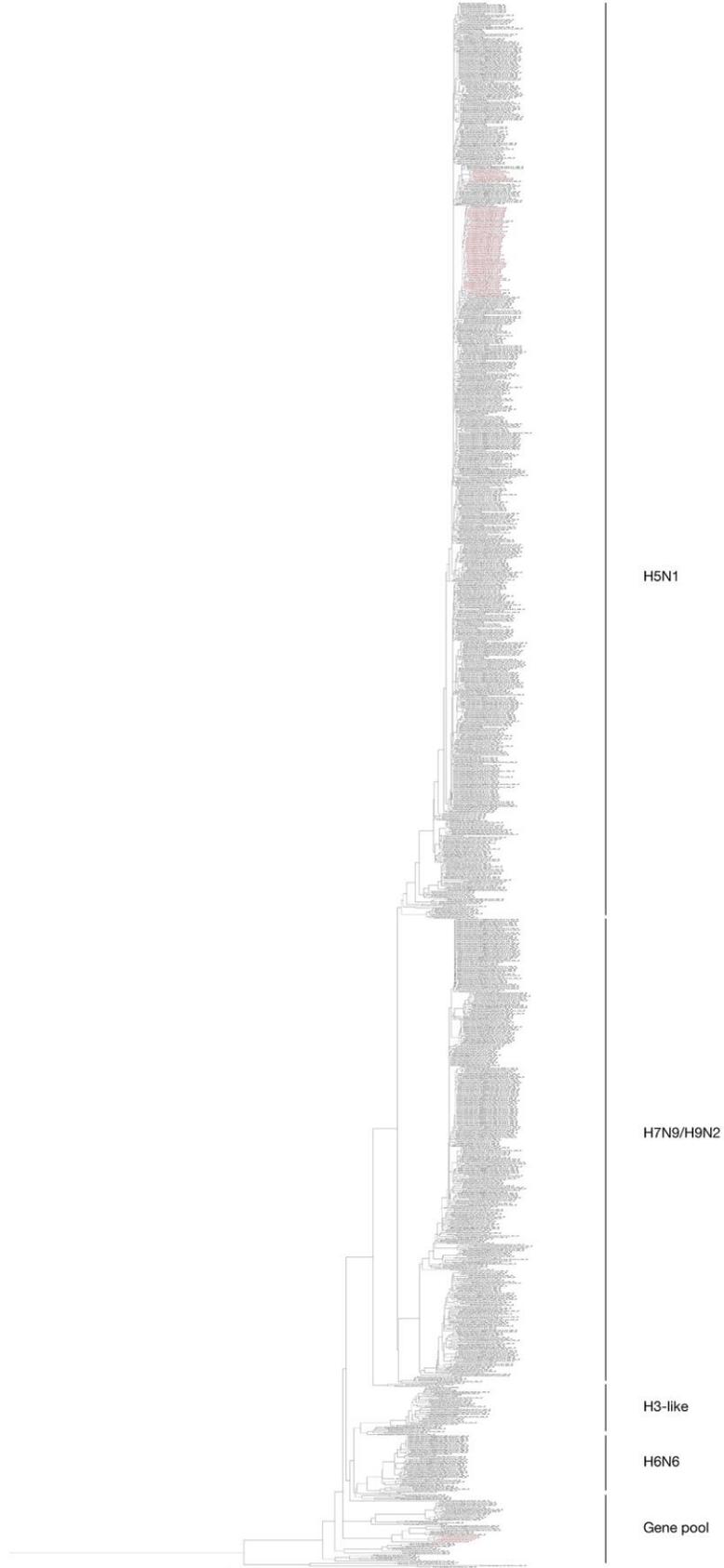
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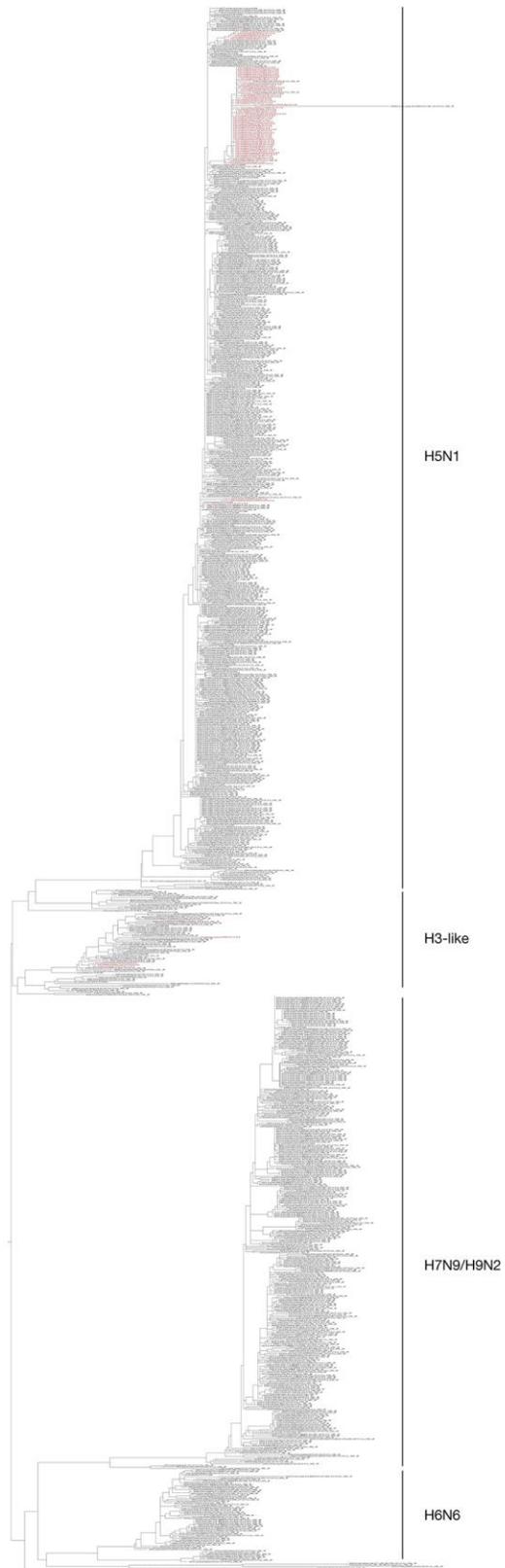
E



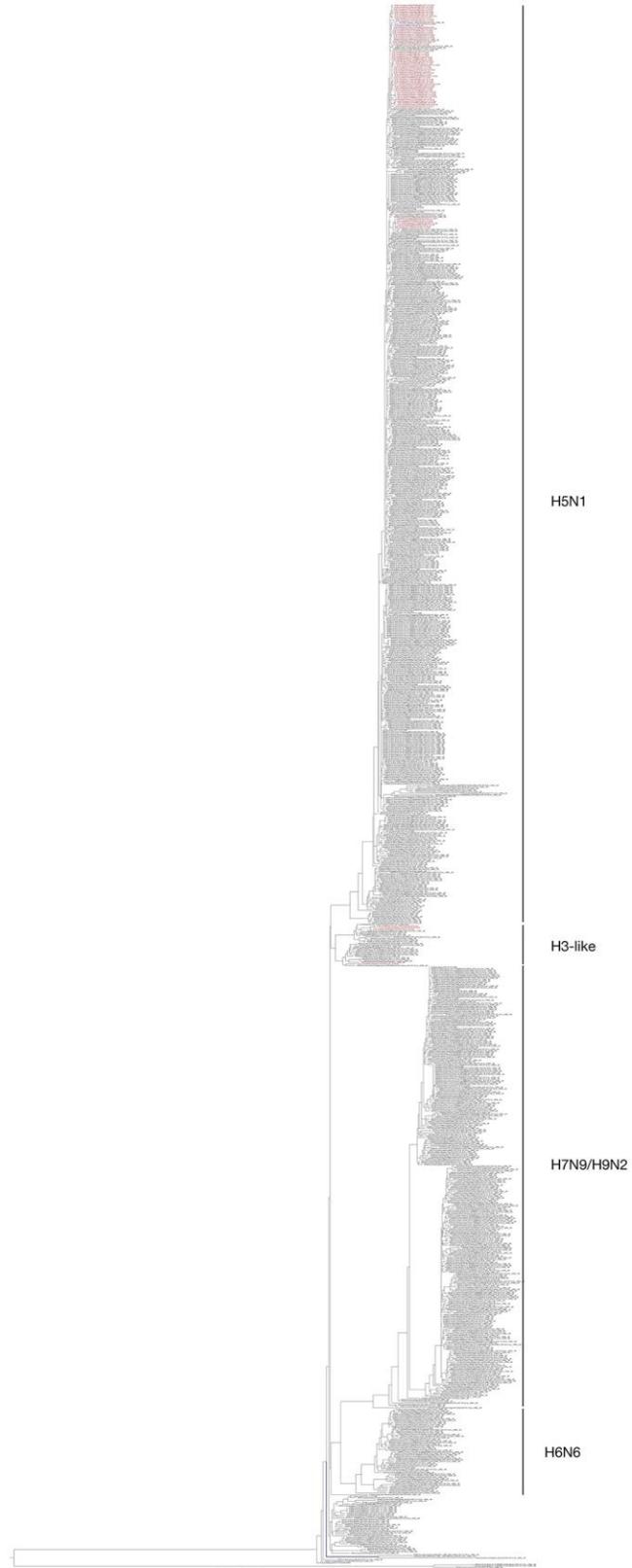
F



G



H



Appendix 1 Figure. Maximum-likelihood phylogenetic trees for the HA, NA, PB2, PB1, PA, NP, M, and NS genes of A(H5N6) avian influenza viruses. The viruses from the environment in this study are highlighted in red; the human case is highlighted in blue. HA, hemagglutinin; M, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB, polymerase basic.